

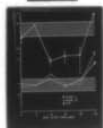
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SUPPRESSION OF PHA-STIMULATED LYMPHOCYTE TRANSFORMATION IN CYN0--ETC(U)  
APR 78 R A KISHIMOTO, J C GONDER

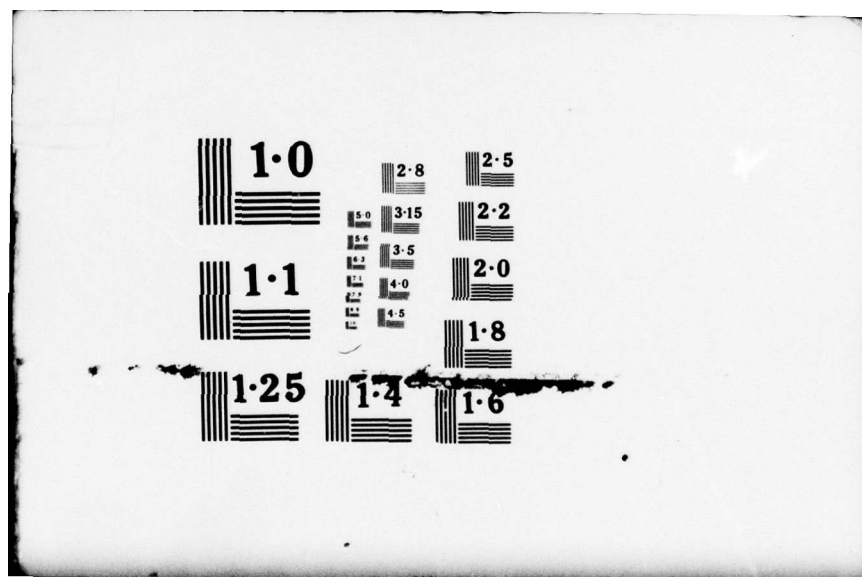
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Suppression of PHA-Stimulated Lymphocyte Transformation  
in Cynomolgus Monkeys Following Infection with Coxiella burnetii

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Running Head: LT SUPPRESSION AFTER Q FEVER

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on the Revision of the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Research Council. The facilities are fully accredited by the American Association for Accreditation of Laboratory Animal Care.

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11 April 1978

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## ABSTRACT

✓ Phytohemagglutinin-induced blastogenesis of peripheral blood lymphocytes from cynomolgus monkeys infected with Coxiella burnetii was suppressed between 14 and 28 days after infection. Lymphocytes became responsive to PHA again on day 35 with an increase in stimulation index when cultured with specific antigens. In contrast, production of specific humoral antibodies was not diminished during the acute and early convalescent stages of infection. ↗

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Several investigators have reported a suppression of lymphocyte blastogenesis during acute infection with various microorganisms (5,6, 8,12). Previous studies have demonstrated a role for humoral and/or cellular immunity in Q fever infections (9-11).

This study was undertaken to examine the cellular and humoral immune responses following infection of cynomolgus monkeys with Coxiella burnetii. The functional activity of T and B lymphocytes was measured by in vitro proliferative response to phytohemagglutinin (PHA) and specific antigens, and production of specific antibodies, respectively.



## MATERIALS AND METHODS

Preparation of rickettsial stock suspension. The third egg passage of the Henzerling strain of C. burnetii in phase I was grown in chicken embryo cells as previously described (9). The infectivity of the rickettsial suspension was estimated to be  $10^{9.5}$  mouse median intraperitoneal infectious doses (MIPID<sub>50</sub>).

Experimental animals. Six cynomolgus monkeys (Macaca fascicularis) of both sexes, weighing 2.0 to 3.5 kg were used in this study. They were provided with commercial monkey chow and water ad libitum throughout the experimental period.

Exposure of animals. Monkeys were restrained by intramuscular injection of 10 mg/kg ketamine hydrochloride (Parke Davis, Detroit, MI), then exposed to  $10^5$  MIPID<sub>50</sub> of the phase I Henzerling strain of C. burnetii presented as small-particle aerosols using the procedure described by Berendt (1).

Preparation of lymphocytes. Peripheral blood lymphocytes were separated using the technique of Böyum (2). The mononuclear cells at the interface were aspirated and washed twice with RPMI 1640 medium (GIBCO, Grand Island, NY) supplemented with penicillin (200 units/ml), streptomycin (200 µg/ml) and 10% normal cynomolgus monkey serum. Purified lymphocytes were then resuspended in the same medium. Microscopic examination of Wright-stained material showed that 90 to 95% of the cells were lymphocytes and 95 to 98% of these cells were viable by trypan blue exclusion.

Lymphocyte transformation assay. Blast transformation was measured by dispensing 100-µl aliquots of approximately  $1 \times 10^5$  lymphocytes into six replicate wells of a U-well microtiter plate (Cooke Laboratory

Products, Alexandria, VA). Then 25  $\mu$ l of one of the following components was added: (i) RPMI medium, (ii)  $2 \times 10^7$  formalin-killed phase I C. burnetii (EP-3 Henzerling strain) in RPMI medium; (iii)  $4 \times 10^6$  phase II (EP-88 Nine-mile strain) formalin-killed rickettsiae in RPMI medium, or (iv) 1:10 dilution of phytohemagglutinin P (PHA) (Difco Laboratories, Detroit, MI). The cells were incubated for 4 days; 0.02  $\mu$ Ci of [ $^{14}$ C]thymidine was added and cells were harvested 24 h later as previously described (11). Lymphocyte transformation (LT) expressed as the stimulation index (SI) was calculated by the following formula:

$$SI = \frac{\text{cpm of culture with PHA or specific antigen}}{\text{cpm of control culture}}$$

All cpm are given as the geometric mean of the six replicate cultures.

Serologic assays. Blood was collected weekly and the serum antibody activity against phase I and II C. burnetii was measured by indirect immunofluorescence (IFA) (3), microagglutination (MA) (7), and complement fixation (CF) (4) tests.

## RESULTS

Following an aerosol exposure to  $10^5$  MIPID<sub>50</sub>, all 6 monkeys developed clinical signs of illness, i.e., fever, increased respiratory rate, cough, anorexia and depression. These signs first appeared between 4 and 7 days after exposure and persisted for 4 to 6 days. Rickettsemia was noted from days 4 to 13. Total leukocyte counts remained within normal limits, but a relative neutrophilia and lymphopenia occurred between 4 and 9 days. By day 10, all hematologic values returned to base-line limits. A report on the clinical findings will be published in an accompanying publication.

Fig. 1. shows the SI of lymphocytes following culture in PHA or phase I or II C. burnetii. Between 14 and 28 days, there was a significant reduction in transformation of lymphocytes by PHA. Stimulation indices returned to base-line values by day 35. A significant response was first observed in lymphocytes cultured in phase I or II C. burnetii on day 35.

The serum antibody titers as measured by the IFA, MA and CF tests are shown in Table 1. Antibody to phase II C. burnetii was initially detected 7 days postexposure and persisted at high levels throughout the experimental period. Specific antibodies detected by the IFA test were consistently higher at every sampling time than the MA or CF results. Phase I antibody was detected on day 14 by the IFA and MA tests, but not detected until day 28 by the CF test.

Suppression of lymphocyte blastogenesis also occurred in monkeys exposed to  $10^3$  MIPID<sub>50</sub> of C. burnetii. Only minimal signs of illness developed; nevertheless, they produced antibodies to phase I and II C. burnetii and suppressed PHA-lymphocyte blastogenesis between 14 and



28 days (data not shown). The stimulation indices of lymphocytes from these monkeys cultured with phase I or II C. burnetii antigens were elevated on day 35 as they were in monkeys infected with  $10^5$  MIPID<sub>50</sub> C. burnetii. This suggests that suppression of blastogenesis occurs even in the absence of severe illness.

#### DISCUSSION

Our studies demonstrate a significant reduction in the degree of transformation of peripheral lymphocytes by PHA during the early convalescent stage of Q fever infection in cynomolgus monkeys. However, lymphocytes exhibited normal blastogenesis on day 7 during overt illness which included lymphopenia. In contrast, suppression of lymphocyte blastogenesis in the presence of PHA occurred between 14 and 28 days when lymphocyte counts were within normal limits.

The transient suppression of lymphocyte transformation has been observed recently with other rickettsiae. Oster found transient suppression of immune responses as measured by LT and delayed type hypersensitivity in guinea pigs infected with several species of spotted fever rickettsiae. (Oster, C.N. Suppression of selected cell-mediated immune responses by spotted fever group rickettsiae. Presented, 17th Interscience Conference on Antimicrobial Agents and Chemotherapy, American Society for Microbiology, New York, Oct 12-14 1977, No. 87).

Lymphocyte suppression occurs only following an acute or mild Q fever infection and not merely antigenic exposure. We have previously shown that peripheral lymphocytes from guinea pigs vaccinated with a killed phase I C. burnetii vaccine had increased stimulation indices when cultured in the presence of PHA (11). In ongoing studies, we have

also observed no LT suppression in cynomolgus monkeys vaccinated with a killed phase I C. burnetii vaccine (unpublished data).

Not all cell-mediated factors are adversely affected by infection. For example, we have demonstrated macrophage migration inhibition to specific antigen during the acute and early convalescent stages of infection (10). We also observed that lymphocyte blastogenesis to PHA was suppressed in guinea pigs during early convalescence, as in monkeys (unpublished data). Therefore, it appears that the proliferative response of lymphocytes is suppressed, but cells are still able to produce other lymphokines such as MIF.

Unlike the suppression of blastogenesis, B-cell function remained intact. Antibody against phase I and II C. burnetii was detected by all three serologic tests.

The cause of lymphocyte suppression observed is not known at this time. It is hoped that further studies will elucidate the mechanism of this finding.

## ACKNOWLEDGMENTS

We thank Robert Stockman and Chester Redmond for their technical assistance.



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TABLE 1. Humoral antibody responses of cynomolgus monkeys following exposure to  $10^5$  C. burnetii presented as small particle aerosol

Day	Reciprocal antibody titer by:					
	IFA		MA		CF	
	Phase I	Phase II	Phase I	Phase II	Phase I	Phase II
-7	0	0	0	0	0	0
7	0	306 <sup>a</sup>	0	7	0	0.5
14	48	2487	19	733	0	55
21	351	7169	470	1070	0	309
28	240	6827	496	1070	6	263
35	405	5461	664	1387	8	389

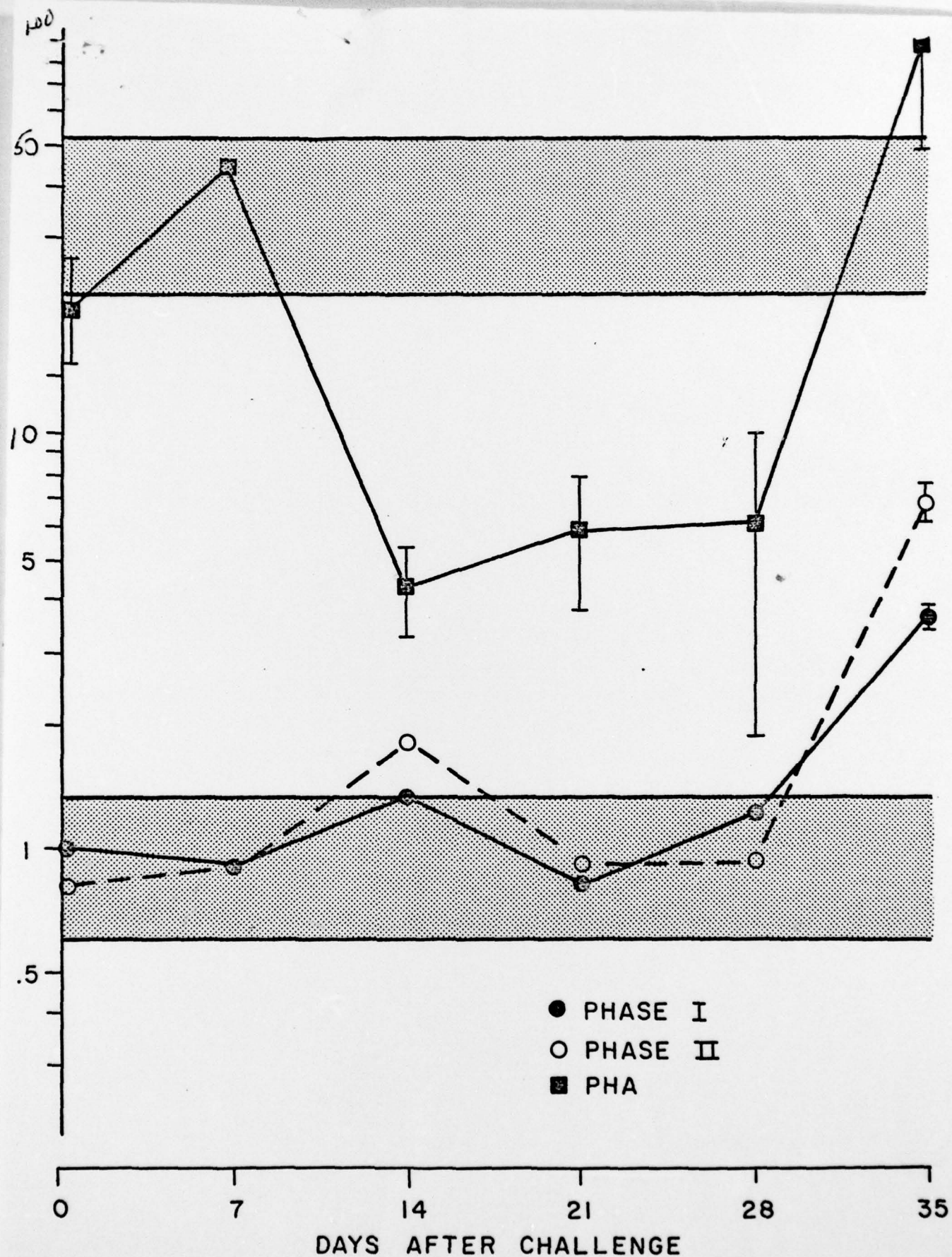
<sup>a</sup>Each point represents the reciprocal geometric mean titer of 6 monkeys.

## FIGURE LEGEND

Fig. 1. Stimulation index of monkeys after challenge with *C. burnetii*.

Shaded bands indicate  $SI + 2 SE$  of normal, noninfected monkeys.

Each point represents the geometric mean + SE obtained from  
6 monkeys.





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REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
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4. TITLE (and Subtitle) SUPPRESSION OF PHA-STIMULATED LYMPHOCYTE TRANS- FORMATION IN CYNOMOLGUS MONKEYS FOLLOWING INFECTION WITH COXIELLA BURNETII.		5. TYPE OF REPORT & PERIOD COVERED Interim rept.
7. AUTHOR(s) R. A. KISHIMOTO J. C. GONDER		6. PERFORMING ORG. REPORT NUMBER
9. PERFORMING ORGANIZATION NAME AND ADDRESS U.S. Army Medical Research Institute of Infectious Diseases SGRD-UIA-E Fort Detrick, Frederick, Maryland 21701		8. CONTRACT OR GRANT NUMBER(s)
11. CONTROLLING OFFICE NAME AND ADDRESS U.S. Army Medical Research and Development Command, Office of the Surgeon General Department of the Army, Frederick, MD 21701		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 3M762776 A841-00-007
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)		12. REPORT DATE 11 Apr 1978
		13. NUMBER OF PAGES 14 12 750
		15. SECURITY CLASS. (of this report) UNCLASSIFIED
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE
16. DISTRIBUTION STATEMENT (of this Report) Cleared for public release; distribution unlimited.		
<div style="border: 1px solid black; padding: 5px; display: inline-block;"> <b>DISTRIBUTION STATEMENT A</b>          Approved for public release;          Distribution Unlimited       </div>		
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report) 16 3M762776A842 17 00		
18. SUPPLEMENTARY NOTES Reprints bearing assigned AD number will be forwarded upon receipt. To be submitted for publication in Infection and Immunity.		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) Phytohemagglutinin Lymphocyte blastogenesis Suppression Q fever		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Phytohemagglutinin-induced blastogenesis of peripheral blood lymphocytes from cynomolgus monkeys infected with <u>C. burnetii</u> was suppressed between 14 and 28 days after infection. Lymphocytes became responsive to PHA again on day 35 with an increase in stimulation index when cultured with specific antigens. In contrast, production of specific humoral antibodies was not diminished during the acute and early convalescent stages of infection.		

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